

Complete classification of one-reaction atoms of multistationarity

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Abstract

This article characterizes certain small multistationary chemical reaction networks. Specifically we identify the ‘smallest CFSTR atoms of multistationarity’, namely those containing one non-flow reaction, which may be irreversible or reversible. We will refer to such atoms as one-reaction atoms of multistationarity. CFSTR atoms of multistationarity were introduced in the recent work of Joshi and Shiu. We recall that a fully open network (alternatively a fully open CFSTR) is a chemical reaction network where all chemical species participate in the inflow and the outflow; and a CFSTR atom of multistationarity N is a multistationary fully open network such that the operation of ‘removing reactions’ or ‘removing species’ destroys the multistationarity of N . The class of CFSTR atoms of multistationarity determines the entire class of multistationary fully open networks since possessing a CFSTR atom of multistationarity as an ‘embedded network’ (N is embedded in G if N can be obtained from G by removing a finite subset of reactions and species) was proven recently to be a sufficient condition for a fully open network to admit multistationarity.

We find that there are infinitely many one-reaction atoms, however the set of such atoms can be characterized using two types, each type containing two parameters. The first type contains one chemical species while the second type contains two chemical species; while both types contain one irreversible reaction. We identify both types with the chemical process of autocatalysis. Furthermore, we give a complete classification by multistationarity of all fully open networks which contain one non-flow (possibly reversible) reaction. Moreover, we obtain new sufficient conditions for establishing multistationarity of certain fully open networks beyond the one-reaction setting.

Keywords: chemical reaction networks, mass-action kinetics, multiple steady states, deficiency one theorem, deficiency one algorithm, atoms of multistationarity.

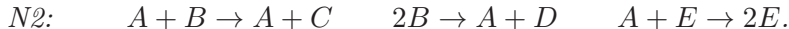
1 Introduction

Chemical reaction networks are used to model systems that occur in chemical engineering and systems biology. The property of existence of multiple positive steady states (also known as multistationarity) provides a necessary condition for a biochemical network to act as a switch. Therefore it is an important problem to determine for which chemical reaction networks there exist positive rate constants for which the network has multiple positive steady states. Determining whether a chemical reaction network admits multiple positive steady states is difficult: for instance, in the mass-action kinetics setting, it requires determining existence of multiple positive solutions to a system of multivariate polynomials with unknown coefficients.

Several criteria exist which help rule out multistationarity in chemical reaction networks. Important examples of such criteria are Deficiency Zero and Deficiency One Theorems of Feinberg [7, 8], the Jacobian criterion and the more general injectivity test of Craciun and Feinberg [1, 2, 3, 4, 11, 13]. Such criteria may be thought of as providing necessary conditions for multistationarity since avoiding these conditions is necessary for multiple steady states. On the other hand, sufficient conditions for multistationarity are relatively rare. Instances where multistationarity can be established include Feinberg’s Deficiency One Algorithm [8] and Ellison and Feinberg’s Advanced Deficiency Algorithm [5]. These criteria have been implemented in the Chemical Reaction Network Toolbox, software available online for free download and use [6].

Within the fully open network setting (a fully open network is a chemical reaction network where all chemical species participate in inflow and outflow), recent results by Joshi and Shiu [12] give a new approach for establishing multistationarity via ‘atoms of multistationarity’ (see Definition 4.9). Possessing an atom of multistationarity as an ‘embedded network’ (see Definition 2.2) is a sufficient condition for multistationarity in fully open networks. Using this approach, the problem of classifying multistationary fully open networks may be reduced to two relatively simpler problems: 1) determining the atoms of multistationarity, and 2) determining whether a network possesses one of the known atoms of multistationarity as an embedded network. Here we focus on the first problem, and provide an answer for the smallest networks.

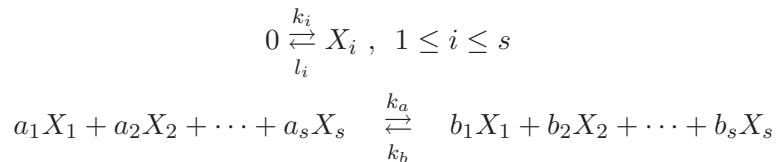
Example 1.1. *Consider the following fully open networks $N1 - N3$ in species A, B, C, D and E . In other words, the flow reactions $0 \rightleftharpoons A$, $0 \rightleftharpoons B$, $0 \rightleftharpoons C$, $0 \rightleftharpoons D$, and $0 \rightleftharpoons E$ are part of all the three networks.*



Which of these three networks admit multiple positive steady states? *Note that $N2$ differs from $N1$ only in the third reaction and in the stoichiometric coefficients of the species E . Moreover, $N2$ differs from $N3$ only in the direction of the first reaction. However, we will show that only $N1$ and $N3$ admit multiple positive steady states (both by virtue of possessing atoms of multistationarity).*

In this work, we characterize the class of ‘smallest’ atoms of multistationarity, namely those containing one non-flow reaction, which may be irreversible or reversible. This is a continuation of the work in [12], where the authors catalog all two-reaction bimolecular atoms of multistationarity. Atoms of multistationarity containing one non-flow reaction will be referred to as *one-reaction atoms of multistationarity*. Consider the following general one-reaction fully

open network consisting of s species all of which are in the inflow and outflow:



where at least one of the rate constants k_a or k_b is assumed to be positive. The k_i and l_i are positive rate constants which indicate the rate at which the species X_i flows in and out, respectively. The stoichiometric coefficients a_i and b_i are assumed to be non-negative integers. The main theorem (Theorem 4.1) in this article gives a simple arithmetic relation on the stoichiometric coefficients which establishes whether the network is multistationary or not.

As corollaries to Theorem 4.1, we get two important results. The first result is Theorem 4.10, which gives a classification of the entire set of one-reaction atoms of multistationarity. We find that the infinitely many one-reaction atoms of multistationarity can be classified into two types, each type parametrized by two integers. The first type contains one chemical species and the second type contains two chemical species. Furthermore, the non-flow reaction in both types of atoms is irreversible. In other words, we find that there are no one-reaction atoms of multistationarity with a reversible non-flow reaction. The second result is Theorem 4.11, which is obtained by combining Theorem 4.1 with the ‘embedded network theorem’ of Joshi and Shiu [12] and extends the applicability of Theorem 4.1 beyond the setting of one-reaction networks. Theorem 4.11 states that a fully open network with any number of non-flow reactions admits multiple steady states if it possesses a one-reaction atom of multistationarity as an embedded network.

We find that the multistationary one-reaction fully open networks including the one-reaction atoms of multistationarity are identified with the chemical process of autocatalysis. Specifically a one-reaction fully open network is multistationary if and only if the network contains a non-flow reaction with a set of species that are autocatalytic (*i.e.* they appear with a higher stoichiometric coefficient in the product complex than in the reactant complex), and the sum of the stoichiometric coefficients in such autocatalytic species in the reactant complex is at least two.

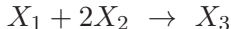
Other authors have previously approached the problem of identifying the smallest chemical reaction networks with a certain specified property. Smallest multistationary chemical reaction networks with the mass-preserving property have been studied in [14]. The smallest chemical reaction outside the fully open network setting (by number of species, number of reactions, and number of terms in the differential equation) was studied in [17] and the smallest chemical reaction network with Hopf bifurcation was studied in [18, 19]. Other examples of classification by multistationarity of small networks include [9, 15, 16]. Recently generalized catalytic and autocatalytic networks have been studied in [10].

This article is organized as follows. Section 2 provides the background information on chemical reaction networks including the basic definitions, notation and the Deficiency One Theorem of Feinberg. Section 3 provides a review of the Deficiency One Algorithm of Feinberg. In Section 4, we state and prove our main theorem which gives a characterization of one-reaction fully open

networks by multistationarity. The first corollary gives a complete classification of one-reaction atoms of multistationarity. As a second corollary we get sufficient conditions for establishing multistationarity of larger networks by way of one-reaction atoms of multistationarity.

2 Chemical reaction network theory

We begin with a review of the notation and basic definitions related to chemical reaction networks. An example of a *chemical reaction* is the following:



The X_i are called chemical *species*, and $X_1 + 2X_2$ and X_3 are called chemical *complexes*. Assigning the *reactant* complex $X_1 + 2X_2$ to the vector $\mathbf{y} = (1, 2, 0)$ and the *product* complex X_3 to the vector $\mathbf{y}' = (0, 0, 1)$, we rewrite the reaction as $\mathbf{y} \rightarrow \mathbf{y}'$. 1, 2 and 0 are respectively the *stoichiometric coefficients* of the species X_1, X_2 and X_3 in the reactant complex $X_1 + 2X_2$. An *autocatalytic reaction* is a chemical reaction in which at least one chemical species which appears in both the product and the reactant complex has a higher stoichiometric coefficient in the product complex than in the reactant complex, for instance $A + B \rightarrow 2A + C$ is autocatalytic. Many of the definitions in this paper follow those in Joshi and Shiu [12]; now we define chemical reaction networks.

Definition 2.1. Let $\mathcal{S} = \{X_i\}$, $\mathcal{C} = \{\mathbf{y}\}$, and $\mathcal{R} = \{\mathbf{y} \rightarrow \mathbf{y}'\}$ denote finite sets of species, complexes, and reactions, respectively. The triple $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is called a chemical reaction network if it satisfies the following:

1. for each complex $\mathbf{y} \in \mathcal{C}$, there exists a reaction in \mathcal{R} for which \mathbf{y} is the reactant complex or \mathbf{y} is the product complex, and
2. for each species $X_i \in \mathcal{S}$, there exists a complex $\mathbf{y} \in \mathcal{C}$ that contains X_i .

A subset of the reactions $\mathcal{R}' \subset \mathcal{R}$ defines the *subnetwork* $\{\mathcal{S}|_{\mathcal{C}|\mathcal{R}'}, \mathcal{C}|\mathcal{R}', \mathcal{R}'\}$, where $\mathcal{C}|\mathcal{R}'$ denotes the set of complexes that appear in the reactions \mathcal{R}' , and $\mathcal{S}|_{\mathcal{C}|\mathcal{R}'}$ denotes the set of species that appear in those complexes. We now define the notion of an embedded network, a more general notion than a subnetwork.

Definition 2.2. Let $\mathfrak{G} = \{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be a chemical reaction network.

1. Consider a subset of the species $S \subset \mathcal{S}$, a subset of the complexes $C \subset \mathcal{C}$, and a subset of the reactions $R \subset \mathcal{R}$.
 - The restriction of R to S , denoted by $R|_S$, is the set of reactions obtained by taking the reactions in R and removing all species not in S from the reactant and product complexes. If a trivial reaction (one in which the reactant and product complexes are the same) is obtained in this process, then it is removed. Also removed are extra copies of repeated reactions.

- The restriction of C to R , denoted by $C|_R$, is the set of (reactant and product) complexes of the reactions in R .
- The restriction of S to C , denoted by $S|_C$, is the set of species that are in the complexes in C .

2. The network obtained from \mathfrak{G} by removing a subset of species $\{X_i\} \subset \mathcal{S}$ is the network

$$\left\{ \mathcal{S} \setminus \{X_i\}, \mathcal{C}|_{\mathcal{R}|_{\mathcal{S} \setminus \{X_i\}}}, \mathcal{R}|_{\mathcal{S} \setminus \{X_i\}} \right\} .$$

3. The network obtained from \mathfrak{G} by removing a set of reactions $\{y \rightarrow y'\} \subset \mathcal{R}$ is the subnetwork

$$\left\{ \mathcal{S}|_{\mathcal{C}|_{\mathcal{R} \setminus \{y \rightarrow y'\}}}, \mathcal{C}|_{\mathcal{R} \setminus \{y \rightarrow y'\}}, \mathcal{R} \setminus \{y \rightarrow y'\} \right\} .$$

4. Let $\mathfrak{G} = \{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be a chemical reaction network. An embedded network of \mathfrak{G} , which is defined by a subset of the species, $S = \{X_{i_1}, X_{i_2}, \dots, X_{i_k}\} \subset \mathcal{S}$, and a subset of the reactions, $R = \{R_{j_1}, R_{j_2}, \dots, R_{j_l}\} \subset \mathcal{R}$, that involve all species of S , is the network $(S, \mathcal{C}|_{R|_S}, R|_S)$ consisting of the reactions $R|_S$.

We will use the notation $\hat{\mathbf{e}}_i \in \mathbb{R}^{|\mathcal{S}|}$ for the canonical basis vector whose i th component is 1 and the other components are 0. Accordingly, $\hat{\mathbf{e}}_i$ is the vector corresponding to the complex X_i .

Definition 2.3. 1. A flow reaction contains only one molecule; such a reaction is either an inflow reaction $\mathbf{0} \rightarrow \hat{\mathbf{e}}_i$ or an outflow reaction $\hat{\mathbf{e}}_i \rightarrow \mathbf{0}$. A non-flow reaction is any reaction that is not a flow reaction.

2. A chemical reaction network is a continuous-flow stirred-tank reactor (CFSTR) if it contains all outflow reactions $\hat{\mathbf{e}}_i \rightarrow \mathbf{0}$ (for all $X_i \in \mathcal{S}$) and a CFSTR is a fully open network if it contains all inflow reactions $\mathbf{0} \rightarrow \hat{\mathbf{e}}_i$. We note that a fully open network is referred to as a fully open CFSTR in [12].

3. A one-reaction fully open network is a fully open network with either one irreversible non-flow reaction or one reversible non-flow reaction.

Let x_i represent the concentration of the chemical species X_i and let $\mathbf{x} = (x_1, \dots, x_{|\mathcal{S}|})$. Let $\mathbf{y}_k = (y_{k1}, \dots, y_{k|\mathcal{S}|})$ be the stoichiometric coefficients of the species $(X_1, \dots, X_{|\mathcal{S}|})$ in the reactant complex of the k -th reaction. For a vector of positive reaction rate constants $(\kappa_1, \kappa_2, \dots, \kappa_{|\mathcal{R}|}) \in \mathbb{R}_{>0}^{|\mathcal{R}|}$ we will assume that the reaction rate is determined by mass-action kinetics, or in other words by

$$\kappa_k \mathbf{x}^{\mathbf{y}_k} := \kappa_k x_1^{y_{k1}} x_2^{y_{k2}} \dots x_{|\mathcal{S}|}^{y_{k|\mathcal{S}|}} \quad (1)$$

where by convention $0^0 = 1$.

A chemical reaction network endowed with mass-action kinetics generates the following system of mass-action ODEs:

$$\dot{\mathbf{x}}(t) = \sum_{k=1}^{|\mathcal{R}|} \kappa_k \mathbf{x}(t)^{\mathbf{y}_k} (\mathbf{y}'_k - \mathbf{y}_k) =: \mathbf{f}(\mathbf{x}(t)) . \quad (2)$$

We define the function *sign* on real numbers by

$$\text{sign}(x) = \begin{cases} 1 & \text{if } x > 0 \\ 0 & \text{if } x = 0 \\ -1 & \text{if } x < 0 \end{cases}$$

Definition 2.4. 1. The stoichiometric subspace of a network is the vector space spanned by the reaction vectors of the network, $S := \text{span}(\{\mathbf{y}' - \mathbf{y} \mid \mathbf{y} \rightarrow \mathbf{y}' \in \mathcal{R}\})$. A vector $\mu \in \mathbb{R}^{|\mathcal{S}|}$ is said to be *sign compatible* with the stoichiometric subspace if there exists a $\lambda \in S$ such that $\text{sign}(\mu_i) = \text{sign}(\lambda_i)$, for $1 \leq i \leq |\mathcal{S}|$. Note that (2) implies that a trajectory $\mathbf{x}(t)$ that begins at a positive vector $\mathbf{x}(0) = \mathbf{c}^0 \in \mathbb{R}_{>0}^{|\mathcal{S}|}$ remains in the stoichiometric compatibility class, which we denote by

$$\mathcal{P} := (\mathbf{c}^0 + S) \cap \mathbb{R}_{\geq 0}^{|\mathcal{S}|} , \quad (3)$$

for all positive time; in other words, this set \mathcal{P} is forward-invariant with respect to (2). Two points in the same stoichiometric compatibility class \mathcal{P} are said to be *stoichiometrically compatible*. Note that \mathbf{y} and \mathbf{y}' are stoichiometrically compatible if $\mathbf{y} - \mathbf{y}' \in S$. A subset of all stoichiometrically compatible positive vectors forms a *positive stoichiometric compatibility class*.

2. A concentration vector $\bar{\mathbf{x}} \in \mathbb{R}_{>0}^{|\mathcal{S}|}$ is a (positive) steady state of the system (2) if $\mathbf{f}(\bar{\mathbf{x}}) = \bar{\mathbf{0}}$. A steady state $\bar{\mathbf{x}}$ is *nondegenerate* if $\text{Im } d\mathbf{f}(\bar{\mathbf{x}}) = S$. (Here, “ $d\mathbf{f}(\bar{\mathbf{x}})$ ” is the Jacobian matrix of \mathbf{f} at $\bar{\mathbf{x}}$: the $|\mathcal{S}| \times |\mathcal{S}|$ -matrix whose (i, j) -th entry is equal to the partial derivative $\frac{\partial f_i}{\partial x_j}(\bar{\mathbf{x}})$).

In the case of a fully open network, the reaction vector for the i -th inflow reaction is the i -th canonical basis vector of $\mathbb{R}^{|\mathcal{S}|}$, so the stoichiometric subspace is $S = \mathbb{R}^{|\mathcal{S}|}$. It follows that for a fully open network, the unique stoichiometric compatibility class is the nonnegative orthant: $\mathcal{P} = \mathbb{R}_{\geq 0}^{|\mathcal{S}|}$.

A chemical reaction network can be thought of as a graph whose vertex set is \mathcal{C} and whose edge set is \mathcal{R} . The next few definitions address the graph-related structure of a chemical reaction network.

Definition 2.5. 1. The complexes \mathbf{y} and \mathbf{y}' are *adjacent* if either $\mathbf{y} \rightarrow \mathbf{y}' \in \mathcal{R}$ or $\mathbf{y}' \rightarrow \mathbf{y} \in \mathcal{R}$. A *linkage class* of a network is a connected component of the network.

2. A complex \mathbf{y} is said to *communicate* with the complex \mathbf{y}' if there exists a subset of \mathcal{C} , $\{\mathbf{y}_1, \dots, \mathbf{y}_n, \mathbf{z}_1, \dots, \mathbf{z}_m\}$, such that $(\mathbf{y} \rightarrow \mathbf{y}_1 \rightarrow \mathbf{y}_2 \rightarrow \dots \rightarrow \mathbf{y}_{n-1} \rightarrow \mathbf{y}_n \rightarrow \mathbf{y}')$ and $(\mathbf{y}' \rightarrow$

$\mathbf{z}_1 \rightarrow \mathbf{z}_2 \rightarrow \dots \mathbf{z}_{n-1} \rightarrow \mathbf{z}_n \rightarrow \mathbf{y}$) are reactions in \mathcal{R} . A terminal strong linkage class of the network is a set of complexes within a linkage class which communicate with each other but do not communicate with any complex outside the set.

Definition 2.6. The deficiency of a chemical reaction network denoted by δ is defined to be $\delta = n - l - d$ where n is the number of distinct complexes in the network, l is the number of linkage classes and d is the dimension of the stoichiometric subspace.

The Deficiency Zero Theorem and the Deficiency One Theorem of Feinberg establish that a network with deficiency zero and a certain subclass of networks with deficiency one cannot admit multiple mass-action steady states.

Theorem 2.7 (Deficiency Zero Theorem, Feinberg [7]). Suppose that a chemical reaction network N has deficiency 0 and that each linkage class of N is a terminal strong linkage class. For all positive reaction rate constants, the mass-action ODEs have precisely one steady state in each positive stoichiometric compatibility class.

Theorem 2.8 (Deficiency One Theorem, Feinberg [7]). Consider a chemical reaction network endowed with mass action kinetics, and with l linkage classes, each containing just one terminal strong linkage class. Suppose that the deficiency of the network is δ , that the deficiencies of the individual linkage classes are $\delta_j, j = 1, \dots, l$, and that these numbers satisfy the following conditions:

$$(i) \delta_j \leq 1, \quad 1, \dots, l.$$

$$(ii) \sum_{j=1}^l \delta_j = \delta.$$

Then, for an arbitrary choice of rate constants, the chemical reaction network does not admit multiple steady states within a positive stoichiometric compatibility class.

The Deficiency One Algorithm stated in Section 3 requires the following regularity condition on the network. Most networks arising as models of chemical processes satisfy this regularity condition.

Definition 2.9. A network is considered to be regular if it satisfies the following conditions:

1. The reaction vectors of the network are positively dependent. In other words, there exists a set of positive numbers $\{\alpha_{\mathbf{y}_i \rightarrow \mathbf{y}_j} | \mathbf{y}_i \rightarrow \mathbf{y}_j \in \mathcal{R}\}$ such that $\sum_{\mathcal{R}} \alpha_{\mathbf{y}_i \rightarrow \mathbf{y}_j} (\mathbf{y}_j - \mathbf{y}_i) = \mathbf{0}$.
2. Each linkage class in the network contains just one terminal strong linkage class.
3. For each pair of adjacent complexes $\{\mathbf{y}_i, \mathbf{y}_j\}$ in a terminal strong linkage class of the linkage class T , if both reactions $\mathbf{y}_i \rightarrow \mathbf{y}_j$ and $\mathbf{y}_j \rightarrow \mathbf{y}_i$ are removed, then T disconnects into two linkage classes.

3 A review of the Deficiency One Algorithm

Here we review the Deficiency One Algorithm of Feinberg [8]. The Deficiency One Algorithm takes as input a regular deficiency one network with two or more linkage classes, each of deficiency zero, and determines whether the network permits multiple steady states or not. The algorithm has two variations depending on whether or not the network contains an irreversible reaction. We now describe the two algorithms. In the following we will let $s := |\mathcal{S}|$ denote the number of species in the network, $r := |\mathcal{R}|$ denote the number of reactions in the network and $\mu = (\mu_1, \dots, \mu_s) \in \mathbb{R}^s$.

3.1 Deficiency one algorithm for a network that contains irreversible reactions

Input: A regular deficiency one network with two or more linkage classes, each of deficiency zero and such that there is at least one irreversible reaction in the network.

Step 1. Determine a set of numbers, $\{g_1, g_2, \dots, g_{|C|}\}$ not all zero, such that the following hold:

- (a) $\sum_{i=1}^{|C|} g_i \mathbf{y}_i = \mathbf{0}$.
- (b) The g_i corresponding to complexes in each linkage class sum to zero.
- (c) The g_i corresponding to complexes in each terminal strong linkage class sum to a nonnegative number.

Step 2. For a pair of adjacent complexes \mathbf{y}_p and \mathbf{y}_q in a terminal strong linkage class, remove the reaction arrows between the pair. Because of regularity Condition 3, the linkage class containing this terminal strong linkage class disconnects into two disjoint components. Sum over the g_i associated with the complexes in one of the resulting two components of the linkage class. Write $\mathbf{y}_p \cdot \mu - \mathbf{y}_q \cdot \mu > 0$ (respectively $= 0$, or < 0) depending on whether sum over of g_i is positive (respectively is zero, or is negative). Repeat this step on the original network for every distinct pair of adjacent complexes in all terminal strong linkage classes.

Step 3. Partition the set of reactant complexes in the network into three subsets U , M and L as follows:

- (a) All complexes that do not belong to a terminal strong linkage class are placed in the subset M .
- (b) All complexes in the same terminal strong linkage class are placed in the same subset.

Step 4. For the partition chosen in step 3, for each pair of distinct complexes $\{\mathbf{y}_i, \mathbf{y}_j\}$ in the subset M , write the relation $\mathbf{y}_i \cdot \mu = \mathbf{y}_j \cdot \mu$.

Step 5. For the partition chosen in step 3, do the following.

- (a) For each complex \mathbf{y}_i in U and each complex \mathbf{y}_j in M write $\mathbf{y}_i \cdot \mu > \mathbf{y}_j \cdot \mu$.
- (b) For each complex \mathbf{y}_j in M and each complex \mathbf{y}_k in L write $\mathbf{y}_j \cdot \mu > \mathbf{y}_k \cdot \mu$.
- (c) For each complex \mathbf{y}_i in U and each complex \mathbf{y}_k in L write $\mathbf{y}_i \cdot \mu > \mathbf{y}_k \cdot \mu$.

Step 6. For the partition chosen in step 3, do the following.

- (a) For each adjacent pair of complexes in each terminal strong linkage class contained in U , write the inequality from Step 2.
- (b) For each adjacent pair of complexes in each terminal strong linkage class contained in L , write the inequality from Step 2 with the inequality sign reversed.

Step 7. Gather all the relations obtained in Steps 4-6 which results in the inequality system for the partition chosen in Step 3.

Step 8. Determine if there exists a nonzero vector μ which satisfies the inequality system corresponding to the partition chosen and which is sign compatible with the stoichiometric subspace of the network. If there does exist such a vector then the network admits multiple positive steady states.

Step 9. Return to Step 3 and choose a new partition of the reactant complexes. Repeat Steps 4-8, and if necessary repeat this step.

Output: If there exists a nonzero vector μ which is sign compatible with the stoichiometric subspace of the network and which satisfies the inequality system for any partition chosen in Step 3, then the network admits multiple mass action steady states. Otherwise, no matter what positive rate constants are chosen, the network does not admit multiple mass action steady states.

3.2 Deficiency one algorithm for reversible networks

Input: A regular deficiency one network with two or more linkage classes, each of deficiency zero and such that all reactions in the network are reversible.

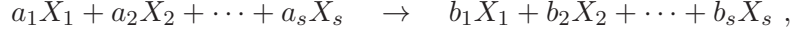
Carry out the same algorithm as the one for a network which contains irreversible reactions. If at the end of the algorithm, multistationarity is not established for the network, then repeat the algorithm with the signs of the g_i chosen in Step 1 reversed.

Output: Same as Section 3.1, establishes multistationarity or otherwise of the regular deficiency one network.

4 Multistationarity in One-Reaction fully open networks

Now we are ready to state our main theorem. The theorem stated below provides a complete characterization by multistationarity of one-reaction fully open networks. The proof of the theorem involves an application of Feinberg's Deficiency One Algorithm [8].

Theorem 4.1. 1. Consider a fully open network endowed with mass action kinetics and which contains only one non-flow reaction:



where $a_i, b_i \geq 0$. Then the fully open network admits multiple positive steady states if and only if the following holds:

$$\sum_{i: b_i > a_i} a_i > 1 . \quad (4)$$

Moreover, these multistationary fully open networks admit nondegenerate steady states.

2. Consider a fully open network endowed with mass action kinetics in which the only non-flow reactions consist of a pair of reversible reactions:



where $a_i, b_i \geq 0$. The fully open network admits multiple positive steady states if and only if the following holds:

$$\sum_{i: b_i > a_i} a_i > 1 \quad \text{or} \quad \sum_{i: a_i > b_i} b_i > 1 . \quad (5)$$

Moreover, these multistationary fully open networks admit nondegenerate steady states.

Remark 4.2. Theorem 4.1 establishes a relation between autocatalysis and multistationarity in one-reaction fully open networks. More precisely, conditions (4) or (5) may be interpreted as follows: a one-reaction fully open network is multistationary if and only if it has an autocatalytic reaction and the total molecularity of the autocatalytic species in the reactant complex is at least two.

Before we prove the theorem, we need a few technical lemmas. In the following two lemmas, we establish the existence of nondegenerate multiple positive mass-action steady states of certain simple but important one-reaction fully open networks.

Lemma 4.3. Let $a_2 > a_1 > 1$. Consider the following fully open network N containing one non-flow reaction:



Let $k^* := \frac{1}{a_2 - a_1} \left(\frac{l_X}{a_1} \right)^{a_1} \left(\frac{a_1 - 1}{k_X} \right)^{a_1 - 1}$. Then the following holds:

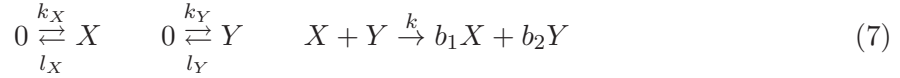
$$\text{If } k \begin{cases} \in (0, k^*) & N \text{ has two nondegenerate positive mass-action steady states} \\ = k^* & N \text{ has one positive mass-action steady state} \\ > k^* & N \text{ has no positive mass-action steady states.} \end{cases}$$

Proof. Let x represent the concentration of the species X . The network (6) when endowed with mass-action kinetics results in the following dynamical system:

$$\dot{x} = f(x) = k_X - l_X x + k(a_2 - a_1)x^{a_1}$$

Clearly $f(0) = k_X > 0$ and $f(x) \rightarrow \infty$ as $x \rightarrow \infty$. Suppose the minimum of $f(x)$ occurs at x^* . Since $f'(x^*) = -l_X + ka_1(a_2 - a_1)(x^*)^{a_1-1} = 0$, we have $x^* = \left(\frac{l_X}{ka_1(a_2 - a_1)}\right)^{\frac{1}{a_1-1}}$ and $f(x^*) = k_X + l_X \left(\frac{1}{a_1} - 1\right) x^*$. If $f(x^*) < 0$ ($= 0, > 0$) then there are two (one, zero resp.) positive steady states. Solving these inequalities for k gives the desired condition. For the case where there are two steady states, the nondegeneracy of the two steady states is clear. \square

Lemma 4.4. *Let $b_1 > 1$ and $b_2 > 1$. The following fully open network M containing one non-flow reaction admits multiple nondegenerate positive mass-action steady states.*



Furthermore, M has two positive nondegenerate mass-action steady states if and only if the parameters satisfy the following inequality:

$$\frac{l_Y}{4k(b_1 - 1)l_X k_X} \left(l_X + \frac{k}{l_Y} (k_X(b_2 - 1) - k_Y(b_1 - 1)) \right)^2 > 1 \quad (8)$$

In the case of equality in the above equation, M has one positive mass-action steady state, and in the case of the reverse inequality M has no positive mass-action steady states.

Proof. Let x and y represent the concentrations of the species X and Y respectively. The network (7) when endowed with mass-action kinetics results in the following dynamical system.

$$\begin{pmatrix} \dot{x} \\ \dot{y} \end{pmatrix} = \begin{pmatrix} f_1(x, y) \\ f_2(x, y) \end{pmatrix} = \begin{pmatrix} k_X - l_X x + k(b_1 - 1)xy \\ k_Y - l_Y y + k(b_2 - 1)xy \end{pmatrix} \quad (9)$$

Some straightforward calculation reveals that the zeros of $f(x, y) := (f_1(x, y), f_2(x, y))$ coincide with the zeros of the following system

$$y = \frac{k_Y}{l_Y} - \frac{1}{l_Y} \left(\frac{b_2 - 1}{b_1 - 1} \right) (k_X - l_X x) \\ g(x) := k_X - \left(l_X + \frac{k}{l_Y} (k_X(b_2 - 1) - k_Y(b_1 - 1)) \right) x + k(b_1 - 1) \frac{l_X}{l_Y} x^2 = 0 \quad (10)$$

So that N has two steady states if and only if the second equation in (10) has two positive roots which occurs when $g(x^*) < 0$ where x^* is the minimum of $g(x)$. The inequality $g(x^*) < 0$ is equivalent to (8). It only remains to show that the set of positive parameters satisfying the inequality (8) is non-empty. To this end, let $k_Y := k_X \left(\frac{b_2 - 1}{b_1 - 1} \right)$, $k := \frac{l_Y}{2(b_1 - 1)l_X}$, and $l_X = k_X + 1$.

With these choices, the left side of (8) is $\frac{(k_X + 1)^2}{2k_X}$ which is greater than 1 for all positive k_X . The nondegeneracy of the two steady states is clear. \square

Remark 4.5. The reaction networks studied in the Lemmas 4.3 and 4.4 are ‘one-reaction atoms of multistationarity’ (see Definition 4.9). The lemmas establish that these one-reaction atoms admit multiple positive nondegenerate mass-action steady states and that the one-reaction atoms admit at most two such steady states. By the theorem of Joshi and Shiu (Lemma 4.7), it follows that any fully open network which contains one of these atoms of multistationarity as an embedded network admits at least two positive nondegenerate mass-action steady states.

Lemma 4.6. Let $\{a_1, a_2, \dots, a_s, b_1, b_2, \dots, b_s\}$ be a set of nonnegative integers. Consider the following system of inequalities:

$$\sum_{i=1}^s a_i \mu_i > \max_{1 \leq j \leq s} \mu_j > 0 \quad (11)$$

$$\text{sign}(\mu_i) = \text{sign}(b_i - a_i) \quad (1 \leq i \leq s). \quad (12)$$

This system has a solution $\mu^* \in \mathbb{R}^s \setminus \{0\}$ if and only if $\sum_{i: b_i > a_i} a_i > 1$.

Proof. We first assume that the inequality system has a nonzero solution denoted by μ^* . So the set $\{j | \mu_j > 0\} = \{j | b_j > a_j\}$ is nonempty and

$$\begin{aligned} \sum_{i: b_i > a_i} a_i \mu_i^* &\geq \sum_{i: b_i > a_i} a_i \mu_i^* + \sum_{i: b_i < a_i} a_i \mu_i^* + \sum_{i: b_i = a_i} a_i \mu_i^* \\ &= \sum_{i=1}^s a_i \mu_i^* > \max_{j: 1 \leq j \leq s} \mu_j^* = \max_{j: b_j > a_j} \mu_j^* > 0. \end{aligned}$$

where in the last line we used (11) twice. This shows that

$$\sum_{i: b_i > a_i} a_i \mu_i^* > \max_{j: b_j > a_j} \mu_j^* \quad (13)$$

If $\sum_{i: b_i > a_i} a_i \leq 1$, then there exists at most one \tilde{i} such that $b_{\tilde{i}} > a_{\tilde{i}} > 0$. So that $\sum_{i: b_i > a_i} a_i \mu_i^* \leq \mu_{\tilde{i}}^*$ which contradicts (13). So we must have $\sum_{i: b_i > a_i} a_i > 1$.

Conversely, assume that $\sum_{i: b_i > a_i} a_i > 1$. For all i such that $b_i > a_i$, choose $\mu_i = 1$ and for all j such that $b_j < a_j$, choose $\mu_j = -\epsilon$ for some $\epsilon > 0$. This choice clearly satisfies (12) and

$$\begin{aligned} \sum_{i=1}^s a_i \mu_i &= \sum_{i: b_i > a_i} a_i \mu_i + \sum_{i: b_i < a_i} a_i \mu_i \\ &= \sum_{i: b_i > a_i} a_i - \epsilon \sum_{i: b_i < a_i} a_i \geq 2 - \epsilon \sum_{i: b_i < a_i} a_i > 1 = \max_{1 \leq j \leq s} \mu_j > 0 \end{aligned}$$

where the last inequality follows by choosing ϵ sufficiently small. \square

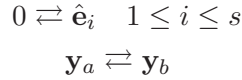
If a fully open network N is an embedded network of a fully open network G , then we can extend the steady states of N to G using the following result.

Lemma 4.7. [Theorem 4.2 and Corollary 4.6 of Joshi and Shiu [12]] Let N be a fully open network embedded in a fully open network G .

- If N admits multiple nondegenerate positive mass-action steady states, then so does G . Moreover, if N admits finitely many such steady states, then G admits at least as many.
- If N admits multiple positive exponentially stable steady states then so does G . Moreover, if N admits finitely many such steady states, then G admits at least as many.

The following lemma deals with some simple cases of one-reaction fully open networks appearing in the statement of Theorem 4.1, which can be handled by using either Deficiency Zero Theorem or Deficiency One Theorem of Feinberg [7].

Lemma 4.8. Consider the following one-reaction fully open network N :



where $\mathbf{y}_b \rightarrow \mathbf{y}_a$ may have a zero reaction rate constant (in other words, the non-flow reaction is possibly irreversible). If $\{\mathbf{y}_a, \mathbf{y}_b\} \cap \{0, \hat{\mathbf{e}}_1, \dots, \hat{\mathbf{e}}_s\} \neq \emptyset$ then N does not admit multiple mass-action steady states.

Proof. We will show that if $\{\mathbf{y}_a, \mathbf{y}_b\} \cap \{0, \hat{\mathbf{e}}_1, \dots, \hat{\mathbf{e}}_s\} \neq \emptyset$ holds then the network has a deficiency of either zero or one. So that either by applying Deficiency Zero or Deficiency One Theorem, multiple mass-action steady states can be ruled out. Since we are assuming that $\mathbf{y}_a \rightarrow \mathbf{y}_b$ is a non-flow reaction, it is not the case that one of the complexes $\{\mathbf{y}_a, \mathbf{y}_b\}$ is the 0 complex and the other is a unimolecular complex. So suppose that one of the complexes (either \mathbf{y}_a or \mathbf{y}_b but not both) in the non-flow reaction is either the 0 complex or is unimolecular. Then the network has only one linkage class ($l = 1$) and $n = s + 2$ complexes so that the deficiency of the network is $\delta = n - l - d = (s + 2) - (1) - (s) = 1$. If the reaction $\mathbf{y}_b \rightarrow \mathbf{y}_a$ has a positive rate constant, then the network is reversible and the entire unique linkage class is a terminal strong linkage class. Otherwise, the unique linkage class has exactly one terminal strong linkage class, either \mathbf{y}_b or $\mathcal{C} \setminus \mathbf{y}_a$, depending on whether $\mathbf{y}_a \in \{0, \hat{\mathbf{e}}_1, \dots, \hat{\mathbf{e}}_s\}$ or $\mathbf{y}_b \in \{0, \hat{\mathbf{e}}_1, \dots, \hat{\mathbf{e}}_s\}$, respectively. In either case, the hypotheses of the Deficiency One Theorem (Theorem 2.8) are satisfied which rules out multiple steady states for such a network.

On the other hand if both \mathbf{y}_a and \mathbf{y}_b are unimolecular, then N has one linkage class ($l = 1$), and $n = s + 1$ complexes. So the deficiency of N is $\delta = n - l - d = (s + 1) - (1) - (s) = 0$. Whether the rate constant for the reaction $\mathbf{y}_b \rightarrow \mathbf{y}_a$ is zero or positive, the entire unique linkage class is a terminal strong linkage class. So by the Deficiency Zero Theorem (Theorem 2.7), N does not admit multiple mass-action steady states. \square

Now we are ready to prove the one-reaction fully open network theorem.

Proof of Theorem 4.1. Assume first that $\{\mathbf{y}_a, \mathbf{y}_b\} \cap \{0, \hat{\mathbf{e}}_1, \dots, \hat{\mathbf{e}}_s\} \neq \emptyset$. This means that at least one of the complexes \mathbf{y}_a or \mathbf{y}_b is at most unimolecular, which implies that $\sum_{i: b_i > a_i} a_i \leq 1$ and

$\sum_{i: a_i > b_i} b_i \leq 1$. Furthermore by Lemma 4.8, the one-reaction network does not admit multiple positive mass-action steady states.

So it suffices to assume that $\{\mathbf{y}_a, \mathbf{y}_b\} \cap \{0, \hat{\mathbf{e}}_1, \dots, \hat{\mathbf{e}}_s\} = \emptyset$. From hereon, we will assume it to be the case that each of \mathbf{y}_a and \mathbf{y}_b is at least bimolecular. In the following we will let $C_a := a_1X_1 + a_2X_2 + \dots + a_sX_s$ and $C_b := b_1X_1 + b_2X_2 + \dots + b_sX_s$ and the corresponding complex vectors are $\mathbf{y}_a = (a_1, \dots, a_s)$ and $\mathbf{y}_b = (b_1, \dots, b_s)$. For the general one-reaction fully open network,

- a. The set of species is $\mathcal{S} := \{X_i | 1 \leq i \leq s\}$.
- b. The set of complexes is $\mathcal{C} = \{\hat{\mathbf{e}}_i | 1 \leq i \leq s\} \cup \{\mathbf{0}\} \cup \{\sum_{i=1}^s a_i \hat{\mathbf{e}}_i, \sum_{i=1}^s b_i \hat{\mathbf{e}}_i\}$. We relabel the complexes $\mathbf{y}_i = \hat{\mathbf{e}}_i$ for $1 \leq i \leq s$, $\mathbf{y}_{s+1} = \mathbf{0}$, $\mathbf{y}_{s+2} = \mathbf{y}_a = (a_1, \dots, a_s)$ and $\mathbf{y}_{s+3} = \mathbf{y}_b = (b_1, \dots, b_s)$.
- c. The set of reactions is either $\mathcal{R}_{irrev} = \{0 \rightleftharpoons \hat{\mathbf{e}}_i | 1 \leq i \leq s\} \cup \{\mathbf{y}_a \rightarrow \mathbf{y}_b\}$ or $\mathcal{R}_{rev} = \{0 \rightleftharpoons \hat{\mathbf{e}}_i | 1 \leq i \leq s\} \cup \{\mathbf{y}_a \rightarrow \mathbf{y}_b\} \cup \{\mathbf{y}_b \rightarrow \mathbf{y}_a\}$ depending on whether the non-flow reaction is irreversible or reversible. The two connected components of the network partition the set of complexes into $\mathcal{C}_1 := \{\hat{\mathbf{e}}_i | 1 \leq i \leq s\} \cup \{\mathbf{0}\}$ and $\mathcal{C}_2 := \{\sum_{i=1}^s a_i \hat{\mathbf{e}}_i, \sum_{i=1}^s b_i \hat{\mathbf{e}}_i\}$.

The proof will proceed by application of the Deficiency One Algorithm. In 1 through 4 in the following, we lay the groundwork for application of the Deficiency One Algorithm by checking the conditions of validity of the algorithm. In 5, we apply the Deficiency One Algorithm to the one-reaction fully open network containing an irreversible non-flow reaction. In 6, we apply the Deficiency One Algorithm to a one-reaction fully open network containing a reversible non-flow reaction, and finally in 7, we establish the nondegeneracy of the steady states for the multistationary fully open networks.

1. **Dimension of the stoichiometric subspace.** For a fully open network, the stoichiometric subspace has ‘full’ dimension. For s species, the dimension of the stoichiometric subspace is $d = s$.
2. **Deficiency of the network.** Since we are assuming that neither of the two complexes in the non-flow reaction is the 0 complex or is unimolecular, $\{\mathbf{y}_a, \mathbf{y}_b\} \cap \{0, \hat{\mathbf{e}}_1, \dots, \hat{\mathbf{e}}_s\} = \emptyset$. The number of complexes is $n = s + 3$, the number of linkage classes is $l = 2$ and the total deficiency is $\delta = n - l - d = (s + 3) - (2) - (s) = 1$. On the other, the deficiency of the two linkage classes is given by

- (a) $\{0 \rightleftharpoons X_i | 1 \leq i \leq s\}$: $\delta_1 = n_1 - 1 - d_1 = (s + 1) - (1) - (s) = 0$.
- (b) For $\{\mathbf{y}_a \rightleftharpoons \mathbf{y}_b\}$: $\delta_2 = n_2 - 1 - d_2 = (2) - (1) - (1) = 0$.

Such networks satisfy the hypotheses of the Deficiency One Algorithm [8] provided they are also regular. In the following we check the regularity of the network.

3. **Regularity of the network.**

- (a) *Regularity Condition 1:* Note that the outflow and inflow reactions for species j have the reaction vectors $\hat{\mathbf{e}}_j$ and $-\hat{\mathbf{e}}_j$. The set $\{\hat{\mathbf{e}}_i, -\hat{\mathbf{e}}_i | i \in \mathcal{S}\}$ forms a basis over the non-negative integers, which implies that the reaction vectors are positively dependent.
- (b) *Regularity Condition 2:* The entire linkage class $\{0, \hat{\mathbf{e}}_1, \dots, \hat{\mathbf{e}}_s\}$ is a terminal strong linkage class. Either the linkage class $\{\mathbf{y}_a, \mathbf{y}_b\}$ is a terminal strong linkage class or contains the terminal strong linkage class $\{\mathbf{y}_b\}$ depending on whether the non-flow reaction is reversible or not.
- (c) *Regularity Condition 3:* If the inflow and outflow reactions of some species j are removed or if the non-flow reaction were to be removed, the linkage class containing that reaction will be disconnected into two linkage classes.

This shows that the network under consideration satisfies the hypothesis of the Deficiency One Algorithm.

4. **Sign compatibility with the stoichiometric subspace.** Since the network under consideration is a fully open network, the stoichiometric subspace has “full” dimension. In other words, the stoichiometric subspace $S = \mathbb{R}^s$. Every vector $\mu^* = (\mu_1, \dots, \mu_s)$ is contained in S and therefore is trivially sign compatible with S . Thus it suffices to determine an inequality system for a partition obtained in Step 3 of the algorithm and then to determine if the inequality system has any solution. If there is such a solution for any partition, then the network admits multiple steady states.
5. **Application of the Deficiency One Algorithm to a network containing at least one irreversible reaction.** Suppose first that the non-flow reaction is not reversible. In other words $(\mathbf{y}_b \rightarrow \mathbf{y}_a) \notin \mathcal{R}$. The set of reaction vectors for the network is $\mathcal{R}_{irrev} = \{\hat{\mathbf{e}}_i, -\hat{\mathbf{e}}_i | i \in \mathcal{S}\} \cup \{\sum_{i=1}^s (b_i - a_i) \hat{\mathbf{e}}_i\}$. Let $r := \text{cardinality}(\mathcal{R}) = 2s + 1$.

Step 1. Let

$$\begin{aligned} g_i &:= a_i - b_i, & (1 \leq i \leq s) \\ g_{s+1} &:= \sum_{i=1}^s (b_i - a_i), & g_{s+2} := -1, \quad g_{s+3} := 1. \end{aligned} \quad (14)$$

We now check that the g_i satisfy the three required conditions.

- (a) $\sum_{i=1}^{|C|} g_i \mathbf{y}_i = \sum_{i=1}^s (a_i - b_i) \hat{\mathbf{e}}_i + \left(\sum_{i=1}^s (b_i - a_i) \right) \mathbf{0} + (-1) \sum_{i=1}^s a_i \hat{\mathbf{e}}_i + (1) \sum_{i=1}^s b_i \hat{\mathbf{e}}_i = \mathbf{0}$.
- (b) It is clear that the g_i sum to zero for the two linkage classes $\mathcal{C}_1 = \{\hat{\mathbf{e}}_i | 1 \leq i \leq s\} \cup \{\mathbf{0}\}$ and $\mathcal{C}_2 = \{\sum_{i=1}^s a_i \hat{\mathbf{e}}_i, \sum_{i=1}^s b_i \hat{\mathbf{e}}_i\}$.
- (c) \mathcal{C}_1 is a terminal strong linkage class while $\{\sum_{i=1}^s b_i \hat{\mathbf{e}}_i\}$ is a terminal strong linkage class within \mathcal{C}_2 . The corresponding g_i sum to 0 and 1, respectively.

Step 2. \mathcal{C}_2 does not contain a pair of complexes in its terminal strong linkage class. In \mathcal{C}_1 , all adjacent pairs of complexes are of the type $\{\mathbf{0}, \hat{\mathbf{e}}_i\}$ for some i . For a fixed

k , let $\mathbf{y}_p := \{\hat{\mathbf{e}}_k\}$ and $\mathbf{y}_q := \mathbf{0}$. So the sum over g_i in \mathbf{y}_p is $g_k = a_k - b_k$ and $\mathbf{y}_p \cdot \mu - \mathbf{y}_q \cdot \mu = \hat{\mathbf{e}}_k \cdot \mu - 0 = \mu_k$. So that for all k we get the following system of inequalities:

$$\text{sign}(\mu_i) = \text{sign}(a_i - b_i) \quad (1 \leq i \leq s) \quad (15)$$

Step 3. The set of reactant complexes is $\mathcal{C} \setminus \{\mathbf{y}_b\}$.

- (a) \mathbf{y}_a is the only complex that does not belong to a terminal strong linkage class and so $\mathbf{y}_a \in M$.
- (b) \mathcal{C}_1 is a terminal strong linkage class and so all the complexes in \mathcal{C}_1 must be placed in the same subset. The three choices of where to place the complexes in \mathcal{C}_1 lead to the following three partitions of the reactant complexes.
 - (i) $U = \emptyset$, $M = \{\mathbf{y}_a\} \cup \{\mathbf{0}\} \cup \{\hat{\mathbf{e}}_i | 1 \leq i \leq s\}$, $L = \emptyset$.
 - (ii) $U = \emptyset$, $M = \{\mathbf{y}_a\}$, $L = \{\mathbf{0}\} \cup \{\hat{\mathbf{e}}_i | 1 \leq i \leq s\}$.
 - (iii) $U = \{\mathbf{0}\} \cup \{\hat{\mathbf{e}}_i | 1 \leq i \leq s\}$, $M = \{\mathbf{y}_a\}$, $L = \emptyset$.

It is straightforward to show that if the inequality system resulting from partition (ii) has a solution μ^* , then the inequality resulting from partition (iii) has a solution which is equal to $-\mu^*$. This is true whenever two partitions are related by switching the contents of the sets U and L [8]. Thus we will restrict attention to partitions (i) and (ii).

Step 4. For partition (i), we get the following system of equations.

$$\begin{aligned} \mu_i &= 0 \quad (1 \leq i \leq s), \quad \sum_{i=1}^s a_i \mu_i = 0, \\ \sum_{i=1}^s a_i \mu_i &= \mu_j \quad (1 \leq j \leq s), \quad \mu_i = \mu_j \quad (1 \leq i, j \leq s) \end{aligned}$$

which clearly does not have a nonzero solution $\mu^* = (\mu_1^*, \dots, \mu_s^*)$. So it suffices to consider partition (ii) only. Beginning here and in all the following steps, we will assume that we are considering partition (ii) even when this is not explicitly stated. For partition (ii), there is only one complex in M and so we do not get any equations from applying this step.

Step 5. Since there is one complex in M and $s + 1$ complexes in L , we get the following system of $s + 1$ inequalities

$$\sum_{i=1}^s a_i \mu_i > 0, \quad \sum_{i=1}^s a_i \mu_i > \mu_j \quad (1 \leq j \leq s)$$

Step 6. For each adjacent pair of complexes in L , we write the inequality from Step 2 with the inequality reversed.

$$\text{sign}(\mu_i) = \text{sign}(b_i - a_i) \quad (1 \leq i \leq s)$$

Step 7. We gather all the inequalities from Steps 4-6 to get the inequality system.

$$\sum_{i=1}^s a_i \mu_i > 0, \quad \sum_{i=1}^s a_i \mu_i > \mu_j \quad (1 \leq j \leq s),$$

$$\text{sign}(\mu_i) = \text{sign}(b_i - a_i) \quad (1 \leq i \leq s).$$

The first inequality holds only if there exists a j such that $\mu_j > 0$. So the first $s + 1$ inequalities are equivalent to $\sum_{i=1}^s a_i \mu_i > \max_{1 \leq j \leq s} \mu_j > 0$. The system of inequalities to be solved may be written as:

$$\sum_{i=1}^s a_i \mu_i > \max_{1 \leq j \leq s} \mu_j > 0$$

$$\text{sign}(\mu_i) = \text{sign}(b_i - a_i), \quad (1 \leq i \leq s) \quad (16)$$

Step 8. By Lemma 4.6 the system of inequalities (16) has a solution if and only if $\sum_{i: b_i > a_i} a_i > 1$.

Step 9. We have already shown in Steps 3 and 4 that we only need to consider partition (ii) since the other two partitions (i) and (iii) do not provide any new information.

This completes the proof of the theorem for networks that contain at least one irreversible reaction.

6. Application of the Deficiency One Algorithm to a reversible network. Now suppose that the non-flow reaction is reversible which results in a reversible network, in other words a network containing only reversible reactions. The set of reaction vectors for the network is $\mathcal{R}_{rev} = \{\hat{\mathbf{e}}_i, -\hat{\mathbf{e}}_i | i \in \mathcal{S}\} \cup \{\sum_{i=1}^s (b_i - a_i) \hat{\mathbf{e}}_i\} \cup \{\sum_{i=1}^s (a_i - b_i) \hat{\mathbf{e}}_i\}$.

The three networks $\mathcal{R}_{rev} = \{0 \rightleftharpoons \hat{\mathbf{e}}_i | 1 \leq i \leq s\} \cup \{\mathbf{y}_a \rightarrow \mathbf{y}_b\} \cup \{\mathbf{y}_b \rightarrow \mathbf{y}_a\}$, $\mathcal{R}_{irrev} = \{0 \rightleftharpoons \hat{\mathbf{e}}_i | 1 \leq i \leq s\} \cup \{\mathbf{y}_a \rightarrow \mathbf{y}_b\}$, and $\tilde{\mathcal{R}}_{irrev} = \{0 \rightleftharpoons \hat{\mathbf{e}}_i | 1 \leq i \leq s\} \cup \{\mathbf{y}_b \rightarrow \mathbf{y}_a\}$ generate the same stoichiometric subspace. By Lemma 4.7, if either \mathcal{R}_{irrev} or $\tilde{\mathcal{R}}_{irrev}$ admits multiple positive nondegenerate mass-action steady states then so does \mathcal{R}_{rev} . This shows that if either $\sum_{i: b_i > a_i} a_i > 1$ or $\sum_{i: a_i > b_i} b_i > 1$, then \mathcal{R}_{rev} admits multiple steady states.

Assume now that $\sum_{i: b_i > a_i} a_i \leq 1$ and $\sum_{i: a_i > b_i} b_i \leq 1$. It only remains to show that the network does not permit multiple steady states. Note that for reversible networks we need to go through the steps of the algorithm twice – once for the g_i chosen according to Step 1, and then again for g_i which are negative of those chosen according to Step 1.

Step 1. For $1 \leq i \leq s + 2$, we let g_i be the same as in (14). Since the complexes and the linkage classes are the same as in the irreversible case, there is nothing to check in parts (a) and (b). For part (c), we note that for a reversible network every terminal strong linkage class is a linkage class and so the g_i corresponding to complexes in all terminal strong linkage classes sum to zero.

Step 2. We obtain the same set of inequalities as in (15) for the terminal strong linkage class \mathcal{C}_1 . Further we note that $1 = g_{s+3} > g_{s+2} = -1$, so we get the following system of inequalities:

$$\begin{aligned} \text{sign}(\mu_i) &= \text{sign}(a_i - b_i), \quad 1 \leq i \leq s \\ \sum_{i=1}^s (b_i - a_i)\mu_i &> 0 \end{aligned}$$

Step 3. The only constraint is that all complexes in \mathcal{C}_1 belong to the same subset and all complexes in \mathcal{C}_2 belong to the same subset. Thus there are 9 distinct partitions. However, Remark 4.1.G in Feinberg [8] tells us that we only need to examine partitions for which both subsets U and L are nonempty, since the condition that all terminal strong linkage classes contain more than one complex is satisfied. Furthermore, as in the irreversible case, interchanging contents of the subsets U and L result in equivalent inequality systems in the sense that if one inequality system has a solution then so does the other. Thus we only need to consider the following partition $U = \mathcal{C}_2 = \{\sum_{i=1}^s a_i \hat{\mathbf{e}}_i, \sum_{i=1}^s b_i \hat{\mathbf{e}}_i\}$, $M = \emptyset$, $L = \mathcal{C}_1 = \{\mathbf{0}\} \cup \{\hat{\mathbf{e}}_i | 1 \leq i \leq s\}$.

Step 4. Since $M = \emptyset$, no equations result from this step.

Step 5. Comparing complexes in U and L we get the following system

$$\begin{aligned} \sum_{i=1}^s a_i \mu_i &> 0, \quad \sum_{i=1}^s a_i \mu_i > \mu_j \quad (1 \leq j \leq s) \\ \sum_{i=1}^s b_i \mu_i &> 0, \quad \sum_{i=1}^s b_i \mu_i > \mu_j \quad (1 \leq j \leq s). \end{aligned}$$

Step 6. We write the inequality from Step 2 with the inequality corresponding to the complex in L reversed.

$$\begin{aligned} \text{sign}(\mu_i) &= \text{sign}(b_i - a_i), \quad 1 \leq i \leq s \\ \sum_{i=1}^s (b_i - a_i)\mu_i &> 0 \end{aligned}$$

Step 7. We gather all the inequalities from Steps 4 to 6.

$$\begin{aligned} \sum_{i=1}^s b_i \mu_i &> \sum_{i=1}^s a_i \mu_i > \max_{1 \leq j \leq s} \mu_j > 0 \\ \text{sign}(\mu_i) &= \text{sign}(b_i - a_i), \quad (1 \leq i \leq s) \end{aligned} \tag{17}$$

Step 8. By Lemma 4.6 this system of inequalities has no nonzero solutions for $\sum_{i: b_i > a_i} a_i \leq 1$.

As mentioned earlier, for the reversible case, we need to carry out the algorithm again with signs of all g_i reversed. This results in an interchange of the roles of a_i and b_i and so it is straightforward to see that we get the following system of inequalities in Step 7.

$$\begin{aligned} \sum_{i=1}^s a_i \mu_i &> \sum_{i=1}^s b_i \mu_i > \max_{1 \leq j \leq s} \mu_j > 0 \\ \text{sign}(\mu_i) &= \text{sign}(a_i - b_i), \quad (1 \leq i \leq s) \end{aligned} \quad (18)$$

Since by hypothesis $\sum_{i: a_i > b_i} b_i \leq 1$, it follows from Lemma 4.6, that the above system of inequalities does not have a nonzero solution. This completes the proof of the theorem for the reversible case.

- 7. Nondegeneracy of the steady states for the multistationary fully open networks.** We have shown that every multistationary one-reaction fully open network has an embedded fully open network of the form satisfying the hypotheses of either Lemma 4.3 or of Lemma 4.4. Since the fully open networks appearing in Lemma 4.3 and Lemma 4.4 admit multiple positive nondegenerate mass-action steady states, by Lemma 4.7, it follows that if one of the one-reaction fully open networks admits multiple positive mass-action steady states, then it admits multiple positive nondegenerate mass-action steady states.

This completes the proof of the theorem. \square

We recall the following definition of CFSTR atom of multistationarity of Joshi and Shiu [12].

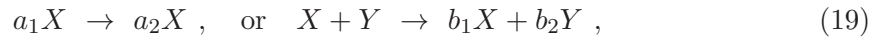
Definition 4.9. 1. *A fully open network is a CFSTR atom of multistationarity if it admits multiple nondegenerate positive mass-action steady states and it is minimal with respect to the embedded network relation among all such fully open networks.*

2. *A one-reaction atom of multistationarity is a CFSTR atom of multistationarity containing one non-flow (irreversible or reversible) reaction.*

3. *A fully open network G is said to possess a CFSTR atom of multistationarity if there exists an embedded network N of G that is a CFSTR atom of multistationarity.*

As a corollary of Theorem 4.1, we get a complete classification of all one-reaction atoms of multistationarity. We state this result as a theorem.

Theorem 4.10. 1. *A one-reaction fully open network is a CFSTR atom of multistationarity if and only if it consists of one irreversible non-flow reaction and that non-flow reaction has one of the following two forms:*

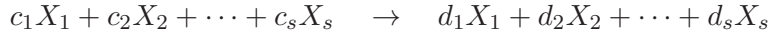


where $a_2 > a_1 > 1$, or, respectively, $b_1 > 1$ and $b_2 > 1$.

2. *A one-reaction fully open network possesses a CFSTR atom of multistationarity as an embedded network if and only if it admits multiple nondegenerate positive mass-action steady states.*

Proof. Evidently, the one-reaction fully open networks in (19) admit multiple nondegenerate positive mass-action steady states by Lemmas 4.3 and 4.4. Furthermore, it is clear that both types of fully open networks are minimal in the class of multistationary fully open networks with respect to the embedded network relation.

On the other hand, assume that N is a one-reaction fully open network with multiple steady states. Then by Theorem 4.1, N has a subnetwork containing a reaction of the following form:



with $\sum_{i:d_i > c_i} c_i > 1$. If there exists a j such that $d_j > c_j > 1$, then we let $X = X_j$, $a_1 = c_j$, and $a_2 = d_j$. Otherwise, there exists a pair of indices (i, j) such that $c_i = c_j = 1$, $d_i > 1$ and $d_j > 1$. In that case, we let $(X, Y) = (X_i, X_j)$, $b_1 = d_i$, and $b_2 = d_j$.

The second part of the theorem follows from Lemma 4.7. This completes the proof. \square

The one-reaction atoms of multistationarity are useful beyond the one-reaction setting. If a one-reaction atom of multistationarity is an embedded network of a fully open network N with possibly more than one non-flow reaction, then the ‘embedding theorem’ of Joshi and Shiu (Lemma 4.7), may be used to infer that N has multiple positive nondegenerate mass-action steady states. We state as a theorem the following important corollary of Theorem 4.1, whose scope of application is beyond the one-reaction setting.

Theorem 4.11. *A fully open network (which may contain more than one non-flow reaction) admits multiple nondegenerate positive mass-action steady states if it contains as an embedded network a reaction of one of the following forms:*

1. $a_1X \rightarrow a_2X$ with $a_2 > a_1 > 1$.
2. $X + Y \rightarrow b_1X + b_2Y$ with $b_1 > 1$ and $b_2 > 1$.

Recall from [12] that bimolecular networks are such that each complex in the network has at most two molecules. In other words, a complex in a bimolecular network has one of the following forms: 0 , A , $2A$ or $A + B$. Theorem 4.1 establishes that there are no bimolecular one-reaction fully open networks with multiple steady states. This in turn implies that the smallest (by number of reactions) bimolecular fully open networks with multiple steady states should contain at least two non-flow reactions (where one reaction is not merely the reverse of the other reaction). In fact, there do exist bimolecular two-reaction CFSTR atoms of multistationarity and this set has been fully catalogued in [12].

We end by posing the question of identifying and characterizing ‘larger’ CFSTR atoms of multistationarity, *i.e.* the ones that contain more than one non-flow reaction. Since tests that involve checking whether a certain large atom of multistationarity is embedded in a network may be computationally difficult, characterization of the CFSTR atoms of multistationarity in terms of general principles may be particularly helpful.

Example 4.12 (Example 1.1 continued). *We end by answering the question posed in Example 1.1.*

1. *For network N1 the fully open network G1 containing the non-flow reaction $2E \rightarrow 3E$ is an embedded network. Since G1 is a one-reaction atom of multistationarity, it follows that N1 is multistationary.*
2. *For the fully open network N3, first remove the reaction $A + E \rightarrow 2E$, and then remove the species C and D. This gives the fully open network G3 containing the non-flow reactions: $A \rightarrow A + B$ and $2B \rightarrow A$. G3 is one of the known two-reaction bimolecular atoms of multistationarity [12] and therefore N3 is multistationary.*
3. *A little bit of calculation shows that N2 possesses no known atoms of multistationarity. In fact, plugging the fully open network N2 into the Chemical Reaction Network Toolbox [6] reveals that N2 does not admit multiple positive steady states.*

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